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European regulatory requirements for veterinary vaccine safety and potency testing and recent progress towards reducing animal use

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Abstract

European technical requirements for veterinary vaccines are laid down in Annex 1, Title II, to Directive 2001/82/EC, as amended by Directive 2009/9/EC, and the European Pharmacopoeia (Ph. Eur.). Safety tests carried out on each batch are generally overdosage studies carried out in at least one of the most sensitive target species and by at least the recommended route of administration that poses the greatest risk. The dose administered should preferably be twice the standard dose for inactivated vaccines and ten times the standard dose for live vaccines. Each batch must also be tested to show that it will contain the appropriate potency or titer to ensure its safety and efficacy. Live vaccines are usually tested by in vitro titration, while serological or challenge tests in vaccinated animals are commonly used for inactivated vaccines, although alternative methods are encouraged if satisfactorily validated. Several amendments have been introduced into the Ph. Eur. to facilitate reduction in the severity of tests and the numbers of animals used, including: the ability to waive the batch safety test when consistency of production has been established; *in vitro* methods to test for extraneous viruses in live poultry vaccines; and humane endpoints for rabies vaccine potency tests. This report discusses some preliminary conclusions concerning how these changes have affected the numbers of animals used during batch control testing of vaccines released via the UK batch release scheme.

Keywords: veterinary vaccines, batch testing, regulations

1. Authorization of veterinary vaccines in the EU

In Europe, medicinal products, including human and veterinary vaccines, may be regulated either by the European Medicines Agency (EMA) or by the various national regulatory authorities. Applications must be made to

[#] The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods and the Interagency Coordinating Committee on the Validation of Alternative Methods

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the EMA for vaccines developed by biotechnology and, optionally, for products that are considered to be novel or subject to Community prophylactic measures. Authorizations are issued by the European Commission and are valid throughout the EC (centrally authorized). All other vaccines are regulated by national authorities who issue authorizations valid only for the respective member state. There are specific procedures for member states to recognize authorizations already issued in other member states and for coordinated assessment of new applications.

European regulatory requirements for medicinal products are governed by Regulation (EC) No 726/2004 (centrally authorized products) [1], Directive 2001/82/EC [2] (nationally authorized products for veterinary use), and Directive 2001/83/EC [3] (nationally authorized products for human use). The technical requirements for veterinary vaccines are laid down in Annex 1, Title II, to Directive 2001/82/EC [2] as amended by Directive 2009/9/EC [4]. Annex 1 also includes a requirement for vaccines to comply with the requirements of the European Pharmacopoeia (Ph. Eur.) [5]. The Ph. Eur. is published by the European Directorate for the Quality of Medicines and Healthcare (EDQM) of the Council of Europe. It is composed of monographs and other texts that define quality standards applicable to medicinal products, including vaccines, in Europe.

2. Safety tests

Both Annex 1 [4] and the Ph. Eur. [5] detail specific requirements for safety testing of veterinary vaccines. These tests should preferably be overdosage studies carried out in at least one of the most sensitive target species and by at least the recommended route of administration posing the greatest risk. The dose administered is usually twice the standard dose for inactivated vaccines and 10 times the standard dose for live vaccines.

Routine application of the batch safety test may be waived in the interests of animal welfare when a sufficient number of consecutive production batches, usually 10, have been produced and found to comply with the requirements. Applications to waive the batch safety test must be made to each competent authority that has authorized the vaccine. The types of data that must be submitted in support of such an application are summarized in the EMA Committee for Veterinary Medicinal Products (CVMP) position paper, “Data Requirements for Removing the Target Animal Batch Safety Test for Immunological Veterinary Medicinal Products in the EU” [6]. These include batch protocol data on at least 10 consecutive batches from separate final bulks, pharmacovigilance data, and a summary of variation applications submitted during the life of the marketing authorization and any effects these changes may have had on the quality and safety profile of the product. The submission must include an expert report that takes into account the nature of the vaccine and the inherent variability of manufacture of the product, the intrinsic safety margin, and the validation that was undertaken to provide the necessary assurance that the product would always be manufactured to an acceptable level of quality and safety. If the manufacturing process is significantly changed, it may be necessary to reinstate the target animal batch safety test to reestablish consistency.

Several specific vaccine monographs in the Ph. Eur. [5], in particular those for vaccines containing clostridial toxoids, detail additional in-process tests in laboratory animals to check for toxicity of the active substances. These tests may need to be repeated until completion of the toxoiding process is confirmed, and can therefore require the use of large numbers of mice and guinea pigs during vaccine manufacture. If alternative methods could be developed to replace these tests, then this would have a major impact on the total number of animals used. There is also a requirement to use additional animals to test for extraneous agent contamination of live viral vaccines.

3. Potency tests

The requirement for a potency test on each vaccine batch is also specified in Annex 1 [4] and the Ph. Eur. [5] to show that each batch will contain the appropriate potency or titer to ensure its safety and efficacy. Live vaccines are usually tested by titration, which does not require the use of animals, while serological or challenge tests in vaccinated animals are commonly used for inactivated vaccines, although alternative methods are encouraged if satisfactorily validated. The current revision of Annex 1 [4] states:

‘A quantification of the active substance shall be carried out on each batch to show that each batch will contain the appropriate potency or titer to ensure its safety and efficacy’.

4. Regulatory measures intended to reduce or refine animal usage

With implementation of the revised text to Annex 1 in 2009 [4], the previous potency requirement to assay the biological activity of the active substance(s) was changed to a requirement to quantify the active substance(s). This should facilitate the regulatory acceptance of *in vitro* antigen quantification assays for new vaccines and encourage the development of such assays for older vaccines. With this in mind, the CVMP Immunologicals Working Party recently published a reflection paper on "control of the active substance in the finished product for immunological veterinary medicinal products (IVMPs)" [7]. The paper discusses the difficulties associated with validation of *in vivo* potency tests and encourages development of alternative *in vitro* methods.

Several changes have been implemented in the Ph. Eur. [5] to reduce the number and severity of animal tests required for batch testing:

- A provision has been introduced into monograph 0062 (Vaccines for Veterinary Use) to waive the target species batch safety test when a sufficient number of consecutive production batches have been produced and found to comply with the test. This should, in time, result in some species no longer being used at all.
- The Ph. Eur. tests for extraneous agents in live poultry vaccines, previously carried out by serology in chickens, have been changed to a range of *in vitro* tests. (Chapters 2.6.3, 2.6.4, 2.6.5, and 2.6.6 are replaced by chapter 2.6.25.)
- The numbers of fish recommended for safety and efficacy testing in some monographs (e.g., monograph 1521) are reduced compared to the numbers commonly used in the past.
- Sections on the use of humane endpoints have been added to the general monographs on human and veterinary vaccines (monographs 0153 and 0062, respectively) and to the respective rabies vaccine monographs (0216 and 0451).

5. Animal use during batch control testing

Animal use during batch control testing of vaccines released via the UK batch release scheme from 2007 to 2009 is currently being analyzed to identify the tests that require the most animals, where changes could have the most impact on numbers and severity of tests, and with a view to monitoring the impact over time of initiatives to reduce animal use. The following general observations have been made on the basis of preliminary results:

- The main species used in batch control testing are summarized in **Figure 1**. The majority of animals used for batch control testing were mice. Significant numbers of chickens, fish, guinea pigs, and hamsters were also used. Other species accounted for only 7% of the total number of animals used.
- From 2007 to 2009, the number of fish used declined. This can probably be linked to reduced numbers of fish recommended in Ph. Eur. monographs for potency and safety tests. However, during the same period the number of mice used increased.
- The mice were mainly used to test for residual toxicity of clostridial toxoids and rabies potency testing.
- Thirty-nine percent of the animals were used for potency tests and 13% for target species safety tests (**Figure 2**). The remaining 48% were mainly used to test for residual toxicity, primarily of clostridial toxoids. Relatively few chickens were used for extraneous agent testing of live vaccines, and these numbers decreased during the period as companies implemented the *in vitro* methods now recommended by the Ph. Eur.

6. Conclusion

Several changes have been made to the European regulatory framework in recent years with the aim of reducing the number and severity of animal tests required for batch testing. Some of these, such as the change in extraneous agent testing of live poultry vaccines and the numbers of fish recommended for safety and efficacy testing of fish vaccines, are already showing benefits with regard to the number of animals used for these tests.

Preliminary data from the UK batch release scheme have identified testing for residual toxicity (mainly clostridial toxoids) and rabies vaccine potency testing as activities that use particularly large numbers of animals. The introduction of humane endpoints for the rabies vaccine potency test should improve the welfare of animals used for this test.

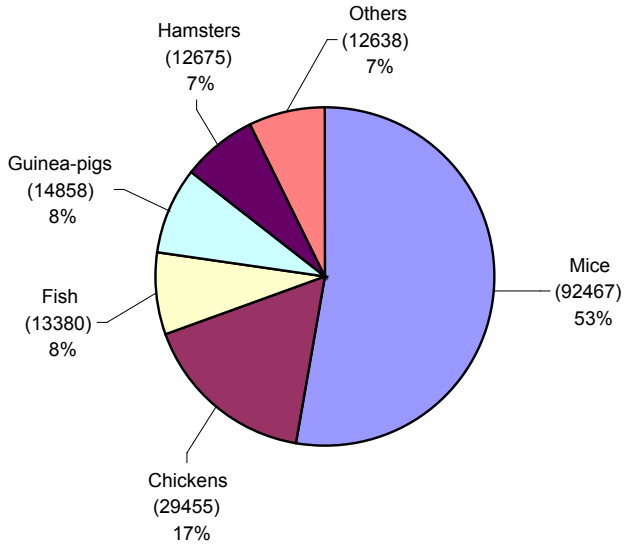

Figure 1. Comparison of species used in batch control testing of veterinary vaccines released in the UK from 2007 to 2009 (preliminary results).

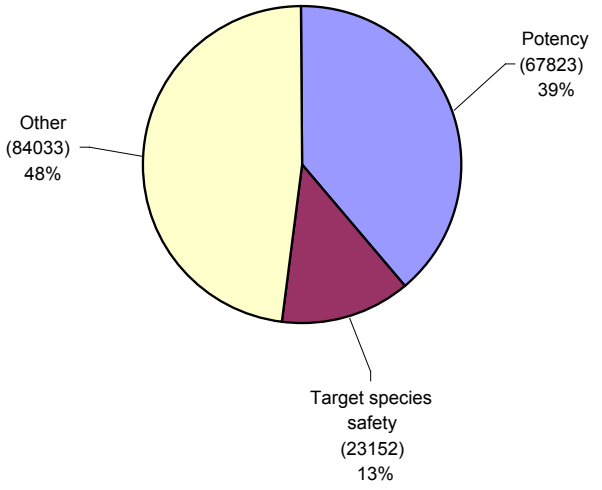

Figure 2. Purpose of animals used in batch control testing of veterinary vaccines released in the UK from 2007 to 2009 (preliminary results). The 'other' tests include tests for residual toxicity and tests for extraneous virus contamination. Acknowledgement

Acknowledgement

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